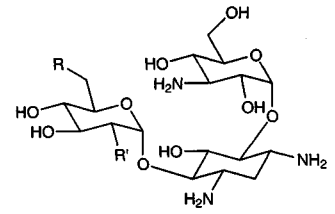
Kanamycin

Molecular formula: $C_{18}H_{36}N_4O_{11}$ (A)
Molecular weight: 484.51 (A)
CAS Registry No.: 8063-07-8, 25389-94-0 (A sulfate), 59-01-8 (A), 4696-78-8 (B)
Merck Index: 5293



	R	R'
Kanamycin A	NH_2	OH
Kanamycin B	NH_2	NH_2
Kanamycin C	OH	NH_2

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. 300 μL Plasma + 30 μL water + 100 μL 2 M perchloric acid, vortex for 2-3 s, centrifuge at 1000 g for 5 min. Remove the supernatant and neutralize it with 1.5 M NaOH, add 300 μL buffer, add 400 μL DMSO, add 100 μL 2% 2,4-dinitrofluorobenzene in EtOH, vortex, heat at 64° for 30 min, add 3 mL toluene, vortex, centrifuge, discard the upper toluene layer, add 3 mL MeCN:toluene 50:50, vortex for 5-10 s. Remove the upper organic layer and evaporate it to dryness under a stream of nitrogen at 30-40°, reconstitute the residue in 1 mL MeCN:water 50:50, inject a 20 μL aliquot. Urine. Dilute urine 100-fold with water. 300 μL Diluted urine + 30 μL water + 300 μL buffer + 400 μL DMSO + 100 μL 2% 2,4-dinitrofluorobenzene in EtOH, vortex, heat at 64° for 30 min, add 3 mL toluene, vortex, centrifuge, discard the upper toluene layer, add 3 mL MeCN:toluene 50:50, vortex for 5-10 s. Remove the upper organic layer and evaporate it to dryness under a stream of nitrogen at 30-40°, reconstitute the residue in 1 mL MeCN:water 50:50, inject a 20 μL aliquot. (Prepare buffer by mixing 80 mL 100 mM Na_2HPO_4 and 20 mL 100 mM NaH_2PO_4, adding 1 g Tris HCl, and adjusting the pH to 7.8 with 6 M HCl.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Zorbax SB-C18
Mobile phase: MeOH:water 64:36, adjusted to pH 3.0 with phosphoric acid
Column temperature: 50
Flow rate: 2
Injection volume: 10-20
Detector: UV 350

CHROMATOGRAM

Retention time: 24.0 (kanamycin B)
Internal standard: kanamycin B (24.0)

OTHER SUBSTANCES

Extracted: paromomycin

KEY WORDS

derivatization; plasma; pharmacokinetics; kanamycin B is IS

REFERENCE

Lu,J.; Cwik,M.; Kanyok,T. Determination of paromomycin in human plasma and urine by reversed-phase high-performance liquid chromatography using 2,4-dinitrofluorobenzene derivatization, *J.Chromatogr.B*, **1997**, *695*, 329–335.

SAMPLE

Matrix: bulk
Sample preparation: Prepare a 2 mg/mL solution in 20 mM pH 9.0 borate buffer, remove a 5 mL aliquot and add it to 15 mL 150 mM 2,4-dinitrofluorobenzene in MeOH (prepare fresh daily), heat at 100° for 45 min, cool, make up to 250 mL with mobile phase, discard the upper aqueous phase, inject a 20 μL aliquot of the lower organic phase.

HPLC VARIABLES

Column: 250 × 4.6 5 μm LiChrosorb SI-100

Mobile phase: Chloroform:THF:water 42:56.4:1.6
Flow rate: 1
Injection volume: 20
Detector: UV 350

CHROMATOGRAM
Retention time: 18

KEY WORDS
normal phase; derivatization

REFERENCE
Tsuji,K.; Goetz,J.F.; VanMeter,W.; Gusciora,K.A. Normal-phase high-performance liquid chromatographic determination of neomycin sulfate derivatized with 1-fluoro-2,4-dinitrobenzene, *J.Chromatogr.*, **1979**, *175*, 141–152.

SAMPLE
Matrix: fermentation solutions
Sample preparation: 5 mL Fermentation broth + 5 mL saturated aqueous solution of Tris + 20 mL MeCN, centrifuge at 3000 rpm for 10 min. Remove a 1 mL aliquot of the supernatant and add it to 3 mL 150 mM 2,4-dinitrofluorobenzene in MeOH, heat at 100° under a reflux condenser for 45 min, make up to 4 mL with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 200 × 4.6 10 μm LiChrosorb RP-8
Mobile phase: MeCN:water:acetic acid 55:45:0.15
Flow rate: 1.2
Injection volume: 20
Detector: UV 350

CHROMATOGRAM
Retention time: 11.28 (kanamycin B)

OTHER SUBSTANCES
Extracted: apramycin, tobramycin

KEY WORDS
derivatization

REFERENCE
Harangi,J.; Deák,M.; Nánási,P.; Bacsa,G. Determination of the major factors of fermentation of the nebramycin complex by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1984**, *7*, 83–93.

SAMPLE
Matrix: formulations
Sample preparation: Dilute 3 mg/mL ophthalmic suspension in water with 10 mM sulfuric acid to a tobramycin concentration of 240 μg/mL. Mix 4 mL diluted suspension with 10 mL 10 mg/mL 2,4-dinitrofluorobenzene in EtOH and 10 mL 15 mg/mL tris(hydroxymethyl)aminomethane in water:dimethylsulfoxide 20:80. Heat at 70 ± 2°. for 20 min, allow to cool slightly for 2 min and add 24 mL MeCN. Allow to cool to room temperature, make up to 50 mL with MeCN, inject a 30 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 Nova-Pak C18
Mobile phase: MeCN:buffer 55:45 (Prepare mobile phase as follows. Dissolve 2.0 g tris (hydroxymethyl)aminomethane in 960 mL water, add 20 mL 0.5 M sulfuric acid and 1200 mL MeCN.)
Flow rate: 1.5
Injection volume: 30
Detector: UV 365

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: neamine, nebramine, tobramycin

KEY WORDS
derivatization; ophthalmic suspension

REFERENCE
Russ,H.; McCleary,D.; Katimy,R.; Montana,J.L.; Miller,R.B.; Krishnamoorthy,R.; Davis,C.W. Development and validation of a stability-indicating HPLC method for the determination of tobramycin and its related substances in an ophthalmic suspension, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 2165–2181.

SAMPLE
Matrix: reaction mixtures
Sample preparation: 50 μL Buffered reaction mixture + 50 μL isopropanol + 50 μL reagent, heat at 60° for 10 min, centrifuge at 1000 g for 2 min, immediately inject a 50 μL aliquot of the supernatant. (Reagent was 80 mM o-phthalaldehyde and 250 mM thioglycolic acid in 1 M boric acid, pH adjusted to 10.4 with 40% KOH.)

HPLC VARIABLES
Column: 100 × 5 Hypersil ODS
Mobile phase: A was MeOH:water:acetic acid 50:45:5 containing 5 g/L heptanesulfonic acid. B was MeOH:water:acetic acid 75:20:5 containing 5 g/L heptanesulfonic acid. A:B 60:40.
Flow rate: 2
Injection volume: 50
Detector: UV 330

CHROMATOGRAM
Retention time: 19 (kanamycin A)

KEY WORDS
derivatization

REFERENCE
Lovering,A.M.; White,L.O.; Reeves,D.S. Identification of aminoglycoside-acetylating enzymes by high-pressure liquid chromatographic determination of their reaction products, *Antimicrob.Agents Chemother.*, **1984**, *26*, 10–12.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeCN, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere octyl
Mobile phase: MeCN:20 mM buffer 52:48 (Buffer was 2.68 g KH_2PO_4 in 1 L water adjusted to pH 3.0 with phosphoric acid.)
Column temperature: 50
Flow rate: 2
Injection volume: 50
Detector: UV 340

CHROMATOGRAM
Retention time: 12

OTHER SUBSTANCES
Simultaneous: amikacin
Noninterfering: acetaminophen, acetazolamide, N-acetylprocainamide, amobarbital, ampicillin, amitriptyline, caffeine, cefamandole, cefoxime, cefoxitin, cephalothin, clindamycin, chloram-

phenicol, chlordiazepoxide, diazepam, erythromycin, ethosuximide, gentamicin, nitrofurantoin, penicillin G, pentobarbital, phenobarbital, phenytoin, primidone, procainamide, quinidine, salicylic acid, secobarbital, tetracycline, theophylline, tobramycin, vancomycin

REFERENCE

Kabra,P.M.; Bhatnager,P.K.; Nelson,M.A. Liquid chromatographic determination of amikacin in serum with spectrophotometric detection, *J.Chromatogr.*, **1984**, *307*, 224–229.

SAMPLE

Matrix: solutions

Sample preparation: 50 µL Buffer solution + 25 µL 242 mg/mL pH 10.4 Tris buffer + 100 µL MeCN:water 50:50 + 30 µL 250 mg/mL 2,4,6-trinitrobenzenesulfonic acid in MeCN:water 80:20, vortex for 10 s, heat at 70° for 15 min, add 2 mL chloroform, shake horizontally at 180 cycles/min for 5 min, centrifuge at 750 g for 5 min. Remove the lower organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 µL MeCN, vortex, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 µm Ultrasphere octyl
Mobile phase: MeCN:50 mM KH_2PO_4 62:38, pH adjusted to 3.5 with phosphoric acid
Flow rate: 2.5
Injection volume: 20
Detector: UV 340

CHROMATOGRAM

Retention time: 6.4

OTHER SUBSTANCES

Simultaneous: tobramycin

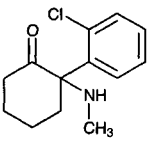
KEY WORDS

derivatization

REFERENCE

Dash,A.K.; Suryanarayanan,R. A liquid-chromatographic method for the determination of tobramycin, *J.Pharm.Biomed.Anal.*, **1991**, *9*, 237–245.

Ketamine



Molecular formula: $C_{13}H_{16}ClNO$
Molecular weight: 237.73
CAS Registry No.: 6740-88-1, 1867-66-9 (HCl)
Merck Index: 5306
Lednicer No.: 1 57

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 20 µL IS + 350 µL 200 mM pH 13 borate buffer, mix. Extract with 5 mL dichloromethane:ethyl acetate 80:20, vortex at 60 rpm for 10 min, centrifuge at 1500g at 15° for 3 min, remove organic phase and extract again with 3 mL dichloromethane:ethyl acetate 80:20. Combine the organic layers and evaporate them to dryness under a stream of nitrogen. Reconstitute the residue in 500 µL dichloromethane:ethyl acetate 80:20, extract with 2 mL 2 M HCl. Remove the aqueous layer and evaporate it to dryness at 45°. Reconstitute the residue in 100 µL mobile phase, inject a 60 µL aliquot.

HPLC VARIABLES

Column: 125 × 4 5 µm Purospher RP-18e (Merck)

Mobile phase: MeCN:30 mM pH 7.2 phosphate buffer 23:77
Column temperature: 20
Flow rate: 1.5
Injection volume: 60
Detector: UV 210

CHROMATOGRAM
Retention time: 16.26
Internal standard: nortilidine (6.29)
Limit of detection: 3 ng
Limit of quantitation: 5 μg/L

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: atropine, buprenorphine, diazepam, dopamide, furosemide, nalbuphine, omeprazole, phenobarbital, phytomenadione, propofol

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Bolze,S.; Boulieu,R. HPLC determination of ketamine, norketamine, and dehydronorketamine in plasma with a high-purity reversed-phase sorbent, *Clin.Chem.*, **1998**, *44*, 560–564.

SAMPLE
Matrix: blood
Sample preparation: Activate two 130 mg Sep-Pak Light C18 SPE cartridges with 1 mL MeOH and 1 mL water. Add 1 mL plasma onto SPE cartridge, wash with 1 mL water, 2 mL 5 mM pH 9.6 ammonium sulfate buffer containing 3% MeCN, and 1 mL 5 mM pH 9.6 ammonium sulfate buffer containing 20% MeCN. Displace washing solution with 200 μL 20 mM pH 2.1 phosphoric acid buffer containing 25% MeCN and elute with 500 μL of the same solution. Mix eluate with 1 mL 40 mM pH 11.5 sodium hydroxide. Add the mixture onto the second SPE cartridge, wash with 2 mL 5 mM pH 9.6 ammonium sulfate buffer containing 3% MeCN and with 1 mL 5 mM pH 9.6 ammonium sulfate buffer containing 20% MeCN. Displace washing solution with 200 μL 20 mM pH 2.1 phosphoric acid buffer containing 25% MeCN and elute with 500 μL of the same solution. Evaporate the eluate in a vacuum centrifuge to about 150 μL, add 12 μL 1 mM sodium hydroxide immediately before injection, inject the whole volume on the column. (Buffers were adjusted with ammonia. SPE flow-rate at all steps was approximately 1.5 mL/min.)

HPLC VARIABLES
Column: 150 × 4.0 5 μm Chiral AGP (Chrom Tech, Sweden)
Mobile phase: MeOH:10 mM pH 7.0 KH_2PO_4 16:84 (Buffer was adjusted with potassium hydroxide.)
Column temperature: 40
Flow rate: 1
Injection volume: 162
Detector: UV 220

CHROMATOGRAM
Retention time: 12 (S), 14 (R)
Limit of detection: 1.7 ng/mL (S), 2.0 ng/mL (R)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolite

KEY WORDS
plasma; SPE; chiral

REFERENCE
Svensson,J.O.; Gustafsson,L.L. Determination of ketamine and norketamine enantiomers in plasma by solid-phase extraction and high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *678*, 373–376.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL bisnortilidine in mobile phase + 100 μL 3 M NaOH, mix, add 5.75 mL ice-cold cyclohexane, agitate at 4° for 15 min, repeat extraction with 3 mL ice-cold cyclohexane. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 500 μL cyclohexane, evaporate to dryness under a stream of nitrogen, reconstitute in 0.2-1 mL mobile phase, mix for 30 s, let stand for 2 min, inject a 50-100 μL aliquot.

HPLC VARIABLES
Column: 100 × 4 AGP (Grom)
Mobile phase: Isopropanol:20 mM pH 7 phosphate buffer 2.5:97.5
Column temperature: 25
Flow rate: 0.5
Injection volume: 50-100
Detector: UV 215

CHROMATOGRAM
Retention time: 22 (S), 26 (R)
Internal standard: bisnortilidine (ethyl trans-2-amino-1-phenyl-3-cyclohexene-1-carboxylatehydrochloride) (one enantiomer only, separated by HPLC) (15)
Limit of detection: 20 ng/mL
Limit of quantitation: 40 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
chiral; plasma; pharmacokinetics

REFERENCE
Geisslinger,G.; Menzel-Soglowek,S.; Kamp,H.-D.; Brune,K. Stereoselective high-performance liquid chromatographic determination of the enantiomers of ketamine and norketamine in plasma, *J.Chromatogr.*, **1991**, *568*, 165–176.

SAMPLE
Matrix: blood
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with water, MeOH, and 100 mM ammonium acetate. Add 200 μL plasma to the SPE cartridge, wash with 100 mM ammonium acetate, elute with MeOH:100 mM ammonium acetate 3:1. Evaporate the eluate to dryness under reduced pressure, dissolve the residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 Hitachi gel 3056 octadecylsilica
Mobile phase: MeOH:100 mM ammonium acetate 60:40
Flow rate: 1
Injection volume: 20
Detector: MS, Hitachi M1000, APCI, nebulizer 260°, vaporizer 399

CHROMATOGRAM
Retention time: 6.2
Limit of detection: 0.5-2.5 ng/mL

OTHER SUBSTANCES
Simultaneous: atipamezole, atropine, butorphanol, flumazenil, medetomidine, midazolam, xylazine

KEY WORDS
plasma; SPE; dog

REFERENCE
Kanazawa,H.; Nagata,Y.; Matsushima,Y.; Takai,N.; Uchiyama,H.; Nishimura,R.; Takeuchi,A. Liquid chromatography-mass spectrometry for the determination of medetomidine and other anaesthetics in plasma, *J.Chromatogr.*, **1993**, *631*, 215–220.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 500 μL MeCN:water 85% phosphoric acid 20:78:2, vortex for 10-15 s, filter (Amicon Centricon-10 microseparation system, 10000 molecular mass cut-off) while centrifuging at 3000 g for 30 min, inject a 20-100 μL aliquot of the ultrafiltrate.

HPLC VARIABLES
Column: 100 × 4.6 3 μm Spherisorb phenyl
Mobile phase: MeCN:MeOH:10 mM NaH_4PO_4:85% phosphoric acid 10:30:59.8:0.2
Column temperature: 50
Injection volume: 20-100
Detector: UV 215

CHROMATOGRAM
Retention time: 5.1
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
horse; serum; ultrafiltrate

REFERENCE
Seay,S.S.; Aucoin,D.P.; Tyczkowska,K.L. Rapid high-performance liquid chromatographic method for the determination of ketamine and its metabolite dehydronorketamine in equine serum, *J.Chromatogr.*, **1993**, *620*, 281–287.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 269

CHROMATOGRAM
Retention time: 4.19
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromet azine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 202.8

CHROMATOGRAM
Retention time: 9.637

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm YMC GEL, ODS-AM coated with poly-(R)-1-(α-naphthyl)ethyl methacrylamide (Prepare (R)-1-(α-naphthyl)ethyl methacrylamide by reacting methacryl chloride with (R)-1-(α-naphthyl)ethylamine. Prepare poly-(R)-1-(α-naphthyl)ethyl methacrylamide by polymerizing this compound in anhydrous benzene/THF with 2,2'-azobis(isobutyronitrile)(Caution! Benzene is a carcinogen!). Average molecular weight = 2500. Coat 4 g 5 μm YMC GEL, ODS-AM with 0.8 g of this polymer using dichloromethane as a solvent.)
Mobile phase: MeCN:0.5M sodium perchlorate 40:60
Flow rate: 1

CHROMATOGRAM
Retention time: k' 3.18 (α = 1.21)

OTHER SUBSTANCES
Also analyzed: propranolol

KEY WORDS
chiral

REFERENCE
Oi,N.; Hashimoto,S.; Ishizuka,N.; Ohtake,J. Enantiomer separation with poly-(R)-1-(α-naphthyl)-ethyl-methacrylamide coated on ODS silica gel by reversed phase HPLC, *Biomed.Chromatogr.*, **1997**, *11*, 296–297.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone,

debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Sumchiral CSP 10 (Sumika Chemical Analysis Service)
Mobile phase: n-Hexane:EtOH:trifluoroacetic acid 200:40:0.6
Flow rate: 1
Detector: UV 230-280

CHROMATOGRAM

Retention time: k' 7.78 (first enantiomer)

KEY WORDS

chiral; α = 1.12

REFERENCE

Oi,N.; Kitahara,H.; Aoki,F. Direct enantiomer separations by high-performance liquid chromatography with chiral urea derivatives as stationary phases, *J.Chromatogr.A*, **1995**, *694*, 129–134.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 CSP-4 (Prepare as follows. Add a solution of 1.07 g L-valyl-L-valyl-L-valine isopropylester (Bunseki Kagaku 1079, 28, 125) in 30 mL dry dioxane (Caution! Dioxane is a carcinogen!) dropwise to a mixture of 2.2 g 2,4,6-trichloro-1,3,5-triazine (cyanuric chloride) in 20 mL dry dioxane stirred at 0°, add 3 g anhydrous sodium carbonate at room temperature, stir, filter, evaporate to give a colorless solid. Dissolve 8.3 g of this solid in 30 mL dry dioxane, add 2 g N-(2-aminoethyl)-3-aminopropyltrimethoxysilane, add 1.5 g anhydrous sodium carbonate, reflux with stirring for 40 h, filter, add 3 g dried 10 μm LiChrosorb Si 100, reflux with slow stirring for 10 h, cool, filter. Wash the solid with dioxane, MeOH, and diethyl ether, dry under reduced pressure (J.Chromatogr. 1984, 292, 427).)
Mobile phase: Hexane:EtOH:trifluoroacetic acid 96:4:0.24
Detector: UV

CHROMATOGRAM

Retention time: k' 7.85 (first enantiomer)

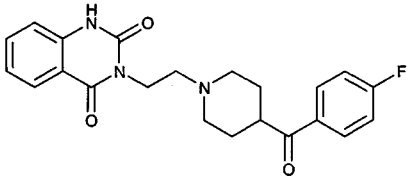
KEY WORDS

chiral; α = 1.09

REFERENCE

Oi,N.; Kitahara,H.; Matsushita,Y.; Kisu,N. Enantiomer separation by gas and high-performance liquid chromatography with tripeptide derivatives as chiral stationary phases, *J.Chromatogr.A*, **1996**, *722*, 229–232.

Ketanserin



Molecular formula: $C_{22}H_{22}FN_3O_3$
Molecular weight: 395.43
CAS Registry No.: 74050-98-9
Merck Index: 5307
Lednicer No.: 3 193

SAMPLE

Matrix: blood
Sample preparation: 25 μL Serum + 50 μL MeOH, vortex for 30 s, centrifuge at 13600 g for 5 min, inject a 25 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: MeCN:buffer:water 31:50:19 (Buffer was 2% acetic acid adjusted to pH 7.0 with ammonium hydroxide.)
Flow rate: 1
Injection volume: 25
Detector: F ex 225 em no emission filter

CHROMATOGRAM

Retention time: 7.7
Limit of quantitation: 40 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

rat; serum; pharmacokinetics

REFERENCE

Wong,Y.W.; Skinner,M.H. Rapid method for the determination of ketanserin in rat serum by high-performance liquid chromatography with fluorimetric detection, *J.Chromatogr.*, **1991**, *571*, 318–323.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.4

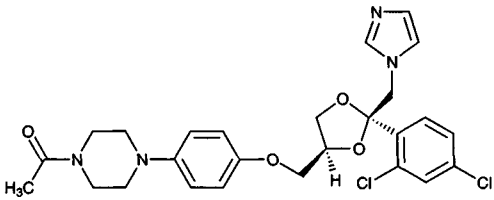
OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Ketoconazole



Molecular formula: $C_{26}H_{28}Cl_2N_4O_4$
Molecular weight: 531.44
CAS Registry No.: 65277-42-1
Merck Index: 5313
Lednicer No.: 3 132

SAMPLE

Matrix: blood
Sample preparation: 500 μL Plasma + 2 μg clotrimazole + hexane:isoamyl alcohol 98.5:1.5, vortex, centrifuge. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 250 μL MeCN, inject an aliquot.

HPLC VARIABLES

Column: 150 × 3.9 NovaPak C18
Mobile phase: MeCN:MeOH:50 mM phosphate buffer 40:5:55
Detector: UV 220

CHROMATOGRAM

Limit of detection: 100-200 ng/mL

KEY WORDS

plasma; pharmacokinetics

REFERENCE

von Moltke,L.L.; Greenblatt,D.J.; Harmatz,J.S.; Duan,S.X.; Harrel,L.M.; Cotreau-Bibbo,M.M.; Pritchard,G.A.; Wright,C.E.; Shader,R.I. Triazolam biotransformation by human liver microsomes in vitro: Effects of metabolic inhibitors and clinical confirmation of a predicted interaction with ketoconazole, *J.Pharmacol.Exp.Ther.*, **1996**, *276*, 370–379.

SAMPLE

Matrix: blood, microsomal incubations
Sample preparation: Vortex 1 mL plasma or microsomal incubation with 200 μL 5 μg/mL diazepam and 100 μL 5 M NaOH solution for 10 s, add 5 mL butan-1-ol:hexane 2:98, vortex for 1 min, centrifuge at 2000 g and 4° for 5 min, evaporate the organic phase to dryness at 40° using a vacuum vortex evaporator, reconstitute the residue in 200 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 5 μm Nova-Pak C18
Mobile phase: MeCN:buffer 35:65 (Buffer was water containing 1% triethylamine, adjusted to pH 3 with orthophosphoric acid.)
Flow rate: 2
Injection volume: 50
Detector: UV 240

CHROMATOGRAM

Retention time: 6.3
Internal standard: diazepam

OTHER SUBSTANCES

Extracted: amitriptyline, nortriptyline
Noninterfering: furafylline, hydroxyamitriptyline, hydroxynortriptyline, quinidine, mephenytoin, triacetyloleandomycin

KEY WORDS

human; liver; rat; plasma

REFERENCE
Ghahramani,P.; Lennard,M.S. Quantitative analysis of amitriptyline and nortriptyline in human plasma and liver microsomal preparations by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *685*, 307–313.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 202.8

CHROMATOGRAM
Retention time: 15.738

KEY WORDS
whole blood

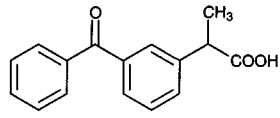
REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 125 × 4 5 μm Hibar RP 18 (Merck)
Mobile phase: MeCN:water:diethylamine 48:55:0.02
Flow rate: 1.2
Injection volume: 20, 100
Detector: UV 254

REFERENCE
Galia,E.; Nicolaides,E.; Hörter,D.; Löbenberg,R.; Reppas,C.; Dressman,J.B. Evaluation of various dissolution media for predicting in vivo performance of class I and II drugs, *Pharm.Res.*, **1998**, *15*, 698–705.

Ketoprofen



Molecular formula: $C_{16}H_{14}O_3$
Molecular weight: 254.29
CAS Registry No.: 22071-15-4
Merck Index: 5316
Lednicer No.: 2 64

SAMPLE

Matrix: blood
Sample preparation: Mix 50 μL plasma with 150 μl 25 μg/mL ibufenac in MeCN:water 95:5, vortex for 30 s, centrifuge for 1 min using a Beckman Microfuge (Beckman Instruments, Palo Alto, CA). Inject a 20 gmL aliquot of the supernatant.

HPLC VARIABLES

Column: 33 × 4.6 3 μm C18 (Perkin Elmer, Norwalk, CT)
Mobile phase: MeCN:buffer 40:60 (Prepare mobile phase as follows. Dissolve 4 mL concentrated phosphoric acid in 600 mL water and mix with 400 mL MeCN.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 220

CHROMATOGRAM

Retention time: 1.1
Internal standard: ibufenac (2.6)
Limit of detection: 1 μg/mL
Limit of quantitation: 5 μg/mL

OTHER SUBSTANCES

Simultaneous: dicloxacillin, ibuprofen
Noninterfering: acetaminophen, N-acetylprocainamide, amikacin, amitriptyline, aspirin, aztreonam, barbituric acid, brompheniramine, cafazolin, caffeine, carbamazepine, carbamazepine epoxide, cephalexin, chlorpheniramine, clonazepam, clotrimazole, desipramine, desmethyldoxepin, digitoxin, digoxin, disopyramide, doxepin, ethosuximide, felbamate, gentamicin, imipenem, imipramine, lidocaine, maprotiline, mephenytoin, mephobarbital, metharbital, methsuximide, methylsuccinimide, nortriptyline, paramethadione, phenacemide, phenobarbital, phensuximide, phenylpropanolamine, phenytoin, primidone, protriptyline, sulfamethoxazole, theophylline, tobramycin, trimethadione, trimethoprim, vancomycin.

KEY WORDS

plasma

REFERENCE

Rifai,N.; Lafi,M.; Sakamoto,M.; Law,T. Measurement of plasma ketoprofen by a rapid high-performance liquid chromatography assay, *Ther.Drug Monit.*, **1997**, *19*, 175–178.

SAMPLE

Matrix: blood
Sample preparation: Condition a 1 mL 100 mg C 18 SPE cartridge (Varian) with 1 mL MeOH and 1 mL 100 mM pH 2.5 phosphate buffer. Mix 100 μL 50 μg/mL 3,4-dimethoxybenzoic acid in MeOH with 1 mL plasma. Vortex with 360 mg ammonium sulfate for 30 min to deproteinate the plasma. Centrifuge at 8000 g at 4°. for 30 min, acidify the supernatant with 3 ml 100 mM sulfuric acid. Add the supernatant to the SPE cartridge, wash twice with 750 μL MeOH:100 mM pH 2.5 phosphate buffer 20:80. Elute with two 500 μL portions of MeOH:50 mM pH 7.4 phosphate buffer 75:25. Inject a 40 μL aliquot.

HPLC VARIABLES

Guard column: Supelco C18

Column: 250 × 4.6 Chirex 3005 [(R)-1-naphtylglycine and 3,5-dinitrobenzoic acid] (Phenomenex, Torrance, CA, USA)
Mobile phase: 20 mM ammonium acetate in MeOH
Flow rate: 1.2
Injection volume: 40
Detector: UV 254

CHROMATOGRAM
Retention time: 14.2 (R-(-)), 16.1 (S-(+))
Internal standard: 3,4-dimethoxybenzoic acid (18.1)
Limit of quantitation: 160 ng/mL

KEY WORDS
SPE; chiral; pharmacokinetics

REFERENCE
Boisvert,J.; Caillé,G.; McGilveray,I.J.; Qureshi,S.A. Quantification of ketoprofen enantiomers in human plasma based on solid-phase extraction and enantioselective column chromatography, *J.Chromatogr.B*, **1997**, *690*, 189–193.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 1 mL 1 M pH 2.0 phosphate buffer + IS, extract with 7 mL diethyl ether, evaporate, reconstitute the residue in 250 μL MeCN:5 mM pH 7.0 phosphate buffer 10:90, inject 50 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 LiChrocart
Column: 125 × 3 5 μm Ecocart packed with LiChrospher 100 RP-18
Mobile phase: MeCN:50 mM pH 7.0 phosphate buffer 16:84
Column temperature: 35
Flow rate: 0.6
Injection volume: 50
Detector: UV 260

CHROMATOGRAM
Retention time: 14.88
Internal standard: naproxen (10.85)
Limit of detection: 30 pg/mL
Limit of quantitation: 100 pg/mL

KEY WORDS
horse; plasma

REFERENCE
Baeyens,W.R.G.; Van Der Weken,G.; Van Overbeke,A.; Corveleyn,S.; Remon,J.P.; Deprez,P. Comparative narrow-bore high-performance liquid chromatographic determination of ketoprofen in horse plasma, *Biomed.Chromatogr.*, **1998**, *12*, 167–169.

SAMPLE
Matrix: blood
Sample preparation: Acidify plasma with an equal volume of 100 mM pH 3.2 phosphoric acid, inject a 20 μL aliquot onto column A and elute to waste with mobile phase A. After 5 min elute the contents of column A onto column B with mobile phase B, monitor the effluent from column B. (Before each run wash column A with MeCN:100 mM pH 3.2 phosphate buffer 20:80 for 3 min, with water for 1 min, with MeOH for 3 min, and with mobile phase A. Equilibrate column B with mobile phase B.)

HPLC VARIABLES
Column: A 10 × 4 5 μm Develosil NH_2-5 (Nomura Chemical Co., Japan) + 10 × 4 Nucleosil 5CN (Macherey-Nagel, Düren, Germany); B 150 × 4.6 Ultron ES-OVM G bonded silica column (Shinwa Kako Co., Japan)

Mobile phase: A 100 mM pH 3.2 phosphate buffer; B MeOH:100 mM pH 3.2 phosphate buffer 20:80
Column temperature: 20
Flow rate: 0.8
Injection volume: 20
Detector: UV 265

CHROMATOGRAM
Retention time: 9.6 (S(+)), 12.0 (R(-))

KEY WORDS
column-switching; chiral; plasma

REFERENCE
Tamai,G.; Edani,M.; Imai,H. Determination of ketoprofen enantiomers in plasma by solid phase extraction and column switching high performance liquid chromatography, *Anal.Sci.*, **1991**, *7*, 29–32.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL tolmetin solution + 500 μL pH 1.8 phosphate buffer, extract with 1-butanol/MTBE. Remove the organic layer and add it to 500 μL pH 6.1 ammonium acetate buffer, mix, inject an aliquot of the aqueous layer.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Cosmosil C18
Mobile phase: MeCN:250 mM pH 5.0 ammonium acetate buffer 20:80
Flow rate: 1.8
Detector: UV 350

CHROMATOGRAM
Internal standard: tolmetin (UV 258)
Limit of quantitation: 5 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Shah,A.K.; Wei,G.; Lanman,R.C.; Bhargava,V.O.; Weir,S.J. Percutaneous absorption of ketoprofen from different anatomical sites in man, *Pharm.Res.*, **1996**, *13*, 168–172.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL Bond-Elut C18 SPE cartridge with 1 mL MeOH. 1 mL Serum + 1 mL water + 20 μL saturated ammonium sulfate solution + 60 μL concentrated HCl, vortex for 3 min, add to the SPE cartridge, wash with three 1 mL portions of water, allow to dry for 3 min, elute with five 500 μL portions of MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 1 mL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Spheri-5 cyano
Mobile phase: MeCN:MeOH:water:phosphoric acid 21:22:56.5:0.5
Flow rate: 0.5
Injection volume: 100
Detector: F ex 248 em 335 (filter) following post-column reaction. The column effluent flowed through a knitted 7.9 m × 0.3 mm ID PTFE coil irradiated with an SC3-9 UV lamp (UVP, San Gabriel CA) and cooled with a fan to the detector.

CHROMATOGRAM
Retention time: 6.5
Internal standard: ketoprofen

OTHER SUBSTANCES
Extracted: fenbufen

KEY WORDS
ketoprofen is IS; post-column reaction; post-column photochemical derivatization; serum; SPE

REFERENCE
Siluveru,M.; Stewart,J.T. Determination of fenbufen and its metabolites in serum by reversed-phase high-performance liquid chromatography using solid-phase extraction and on-line post-column ultraviolet irradiation and fluorescence detection, *J.Chromatogr.B*, **1996**, *682*, 89–94.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 200 μL MeCN, vortex for 30 s, centrifuge at 14000 g for 30 s, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Econosphere CN
Mobile phase: MeCN:water:phosphoric acid 4:100:0.02
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 7
Limit of detection: 100 ng/mL

KEY WORDS
comparison with capillary electrophoresis; serum

REFERENCE
Friedberg,M.; Shihabi,Z.K. Ketoprofen analysis in serum by capillary electrophoresis, *J.Chromatogr.B*, **1997**, *695*, 193–198.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 19.628

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: cell suspensions
Sample preparation: Centrifuge cell suspension at 2000 g for 4 min. Remove a 2 mL aliquot of the supernatant and add it to 200 μL 4 mg/mL IS in DMF, mix, add 200 μL 5 M HCl, extract twice with 3 mL portions of toluene. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 mL dichloromethane, add 20 μL 40 mg/mL 1-hydroxybenzotriazole in dichloromethane:pyridine 99:1, add 300 μL 40 mg/mL 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in dichloromethane, add 300 μL 40 mg/mL (-)-(S)-α-methylbenzylamine in dichloromethane, let stand for 30 min, evaporate to dryness, reconstitute with 500 μL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 10 mm long Techsphere ODS (HPLC Technology, Macclesfield UK)
Column: 250 × 5 5 μm Techsphere ODS (HPLC Technology, Macclesfield UK)
Mobile phase: MeCN:7.5 mM NaH_2PO_4 50:50, containing 5 mM sodium pentanesulfonate, pH adjusted to 2.8 with phosphoric acid
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: k' 6.60, 7.55 (enantiomers)
Internal standard: phenylacetic acid (k' 2.50)
Limit of detection: 1 μg/mL

KEY WORDS
derivatization; chiral

REFERENCE
Thomason,M.J.; Hung,Y.-F.; Rhys-Williams,W.; Hanlon,G.W.; Lloyd,A.W. Indirect enantiomeric separation of 2-arylpropionic acids and structurally related compounds by reversed phase HPLC, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1765–1774.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 250 μL Microsomal incubation, 150 μL ice-cold MeCN and 2.5 ng ketoprofen, centrifuge. Extract the mixture with 4 mL ethyl acetate, centrifuge at 3000 rpm for 10 min, remove the organic fraction and evaporate it under a gentle stream of nitrogen at 40°. Dissolve the residue in 30 μL MeOH and dilute to 60 μL with water. Inject a 30 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm RP-18 (Kanto Chemical, Tokyo)
Mobile phase: MeCN:water 40:60 containing 0.6% acetic acid
Column temperature: 35
Flow rate: 1
Injection volume: 30
Detector: UV 260

CHROMATOGRAM
Retention time: 18.0

OTHER SUBSTANCES
Extracted: metabolites, indomethacin

KEY WORDS
liver; pharmacokinetics; ketoprofen is IS

REFERENCE
Nakajima,M.; Inoue,T.; Shimada,N.; Tokudome,S.; Yamamoto,T.; Kuroiwa,Y. Cytochrome P450 2C9 catalyzes indomethacin O-demethylation in human liver microsomes, *Drug Metab.Dispos.*, **1998**, *26*, 261–266.

SAMPLE
Matrix: permeate

HPLC VARIABLES
Column: 250 × 4 ODS (Hitachi)
Mobile phase: MeCN:50 mM phosphoric acid 40:60 adjusted to pH 5.5 with NaOH
Column temperature: 55
Flow rate: 0.6
Injection volume: 20
Detector: UV 260

OTHER SUBSTANCES
Also analyzed: carbamazepine, fenbufen, indomethacin, α.-naphthoquinone, naproxen, tolmetin

REFERENCE
Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, *87*, 960–966.

SAMPLE
Matrix: solutions
Sample preparation: Mix 50 μL of a 0.001-5 mM solution in MeCN with 50 μL 1 mM DNS-APy in MeCN containing 50 mM 2,2'-dipyridyl disulfide and 50 mM triphenylphosphine, let stand at room temperature for 30 min. Remove a 10 μL aliquot and dilute it to 100 μL with MeCN, inject a 2 μL aliquot. (Synthesis of DNS-APy, 1-(5-dimethylamino-1-naphthalenesulfonyl)-(S)-3-aminopyrrolidine, is as follows. Cool a solution of 16.4 g (R)-(+)-1-benzyl-3-pyrrolidinol in 164 mL pyridine to +5°, add 19.35 g p-toluenesulfonyl chloride, stir at +10° for 48 h, evaporate to dryness, chromatograph using dichloromethane:acetone 95:5 to obtain (3R)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine (mp 68°). Heat a solution of (3R)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine in 200 mL anhydrous DMF to 65°, add 33.5 g sodium azide (Caution! Sodium azide is highly toxic!), stir at 60° for 7 h, filter, evaporate the filtrate to dryness under reduced pressure, dissolve the residue in ethyl acetate, wash twice with water, dry over anhydrous magnesium sulfate, evaporate to obtain (3S)-3-azido-1-(phenylmethyl)pyrrolidine as an oil. Add 3.5 g 10% palladium on carbon under nitrogen to a solution of 7.05 g (3S)-3-azido-1-(phenylmethyl)pyrrolidine in 34.8 mL 1 M HCl in water and 245 mL EtOH, hydrogenate at atmospheric pressure for 30 min, add 3.5 g catalyst, hydrogenate for 2 h, filter, add 34.8 mL 1 M HCl to the filtrate, evaporate to dryness under reduced pressure, take up the residue in 70 mL EtOH, filter, evaporate the filtrate to dryness under reduced pressure, repeat this operation twice, crystallize with the minimum amount of EtOH to obtain (3S)-(+)-3-aminopyrrolidine dihydrochloride (J. Med. Chem. 1992, 35, 4205). (3S)-(+)-Aminopyrrolidine dihydrochloride is also reported to be available from Tokyo Kasei. Stir 800 mg (3S)-(+)-3-aminopyrrolidine dihydrochloride and 2 mL triethylamine in 800 mL MeCN at 0-10°, add a solution of 440 mg dansyl chloride in 80 mL MeCN dropwise, stir in the dark for 30 min, evaporate to dryness under reduced pressure, dissolve the residue in 200 mL 5% HCl, wash twice with 40 mL portions of dichloromethane. Adjust the pH of the organic layer to 13-14 with 5% NaOH, extract twice with 10 mL portions of dichloromethane. Combine the organic layers and wash them with 80 mL water. Dry the organic layer over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, dissolve the residue in dichloromethane:MeOH 90:10, chromatograph on silica gel with dichloromethane:MeOH 90:10. Collect the greenish-yellow fluorescent band and evaporate it under reduced pressure to obtain DNS-APy as a greenish-yellow oil.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm TSK gel ODS-80TM (Tosoh)
Mobile phase: MeCN:water 50:50

Flow rate: 1
Injection volume: 2
Detector: F ex 340 em 530

CHROMATOGRAM
Retention time: 37 ((S)-(+)), 40 ((R)-(-))
Limit of detection: 0.1 pmole

OTHER SUBSTANCES
Simultaneous: pranoprofen

KEY WORDS
derivatization; chiral

REFERENCE
Al-Kindy,S.; Santa,T.; Fukushima,T.; Homma,H.; Imai,K. 1-(5-Dimethylamino-1-naphthalenesulphonyl)-(S)-3-aminopyrrolidine (DNS-Apy) as a fluorescence chiral labelling reagent for carboxylic acid enantiomers, *Biomed.Chromatogr.*, **1997**, *11*, 137–142.

SAMPLE
Matrix: urine
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 10 mL ethyl acetate and dry by aspiration of air. Evaporate an aliquot of 100 μL 20 μg/mL IS in MeOH to dryness at 37°. Add 1 mL urine, vortex, add 250 μL 1 M pH 5.0 acetate buffer, vortex. Add 250 μL of the mixture to the SPE cartridge, dry by aspiration of air, elute with 3 mL ethyl acetate, evaporate the eluate to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 μL mobile phase, inject a 10-30 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeCN:50 mM pH 5.0 phosphate buffer 42:5
Flow rate: 0.9
Injection volume: 10-30
Detector: UV 230

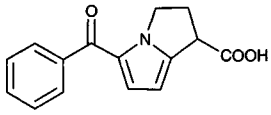
CHROMATOGRAM
Retention time: 6
Internal standard: indomethacin (18.5)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: diclofenac, ibuprofen, felbinac, fenbufen, flurbiprofen, loxoprofen, mefenamic acid, naproxen, piroxicam, sulindac

KEY WORDS
SPE

REFERENCE
Hirai,T.; Matsumoto,S.; Kishi,I. Simultaneous analysis of several non-steroidal anti-inflammatory drugs in human urine by high-performance liquid chromatography with normal solid-phase extraction, *J.Chromatogr.B*, **1997**, *692*, 375–388.

Ketorolac



Molecular formula: $C_{15}H_{13}NO_3$
Molecular weight: 255.27
CAS Registry No.: 74103-06-3, 74103-07-4 (tromethamine)
Merck Index: 5318
Lednicer No.: 4 81

SAMPLE

Matrix: blood
Sample preparation: Dilute 0.1-1 mL plasma to 1 mL with water, add 100 μL 2 μg/mL IS in MeOH:water 90:10, add 100 μL 500 mM pH 3 sodium acetate buffer, add 6 mL hexane:ethyl acetate 70:30, vortex vigorously for 5 min. Centrifuge at 2000 rpm for 2-5 min, place in dry ice/isopropanol or dry ice/MeOH bath to freeze the aqueous layer. Decant the organic layer and evaporate it to dryness under a stream of nitrogen at 38°. Add 500 μL MeOH:water 90:10 and 3 mL hexane, sonicate for 15 s, vortex vigorously for 3 min, let stand for at least 5 min, discard the hexane layer. Evaporate the remaining MeOH/water to dryness under a stream of nitrogen at 38°. Add 100 μL MeOH and 100 μL mobile phase, sonicate for 30 s, vortex for 30 s, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 4 μm Nova Pak C18
Mobile phase: MeCN:0.05% phosphoric acid 34:66
Flow rate: 1
Injection volume: 20
Detector: UV 317

CHROMATOGRAM

Retention time: 5.5
Internal standard: RS-37414-000, (±)-5-p-fluorobenzoyl-1,2-dihydro-3H-pyrrolo[1,2a]-pyrrole-1-carboxylic acid (7)
Limit of quantitation: 10 ng/mL

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Tsina,I.; Chu,F.; Kaloostian,M.; Pettibone,M.; Wu,A. HPLC method for the determination of ketorolac in human plasma, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 957–967.

SAMPLE

Matrix: blood
Sample preparation: Condition a 10 × 3 C18 SPE cartridge (Analytichem) with 2 mL MeOH, 2 mL water, and 2 mL 50 mM pH 3.5 sodium acetate at 2 mL/min. 550 μL Plasma + 550 μL 0/9% NaCl, vortex vigorously, add 25 μL 10 μg/mL ketoprofen in MeOH:water 10:90, add 1 mL to the SPE cartridge at 1 mL/min, wash with 1 mL 50 mM pH 3.5 sodium acetate at 1 mL/min, wash with 1.5 mL MeOH:0.1% acetic acid 20:80 at 1.5 mL/min, elute the contents of the cartridge on to the column with mobile phase.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm Newguard RP-18
Column: 100 × 8 4 μm Nova-pak C18 radial pak
Mobile phase: Gradient. MeCN:0.1% acetic acid from 30:70 to 60:40 over 10 min, maintain at 60:40 for 2 min, to 100:0 over 3 min.
Flow rate: 2
Detector: UV 313 for 7.2 min then UV 258

CHROMATOGRAM

Retention time: 6.7

Internal standard: ketoprofen (8.8)
Limit of quantitation: 5 ng/mL

KEY WORDS
plasma; SPE

REFERENCE
Solà,J.; Pruñonosa,J.; Colom,H.; Peraire,C.; Obach,R. Determination of ketorolac in human plasma by high-performance liquid chromatography after automated on-line solid-phase extraction, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 89–99.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 100 μL 600 mM sulfuric acid + 3 mL isooctane:isopropanol 95:5, vortex for 30 s, centrifuge at 1800 g for 5 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL 2 mg/mL 4-(dimethylamino)pyridine in MeCN, add 100 μL 60 mM trichloroethyl chloroformate in MeCN, add 1 M L-leucinamide in MeCN, let stand for 2 min, add 500 μL 250 mM HCl, extract with chloroform. Remove the organic layer and evaporate it to dryness, reconstitute the residue in mobile phase, inject a 10-100 μL aliquot. (A 7% conversion of S to R is observed during the derivatization procedure. No racemization is observed using a direct procedure with a chiral column.)

HPLC VARIABLES
Column: 100 × 4.6 5 μm Partisil 5 ODS-2
Mobile phase: MeCN:60 mM KH_2PO_4:triethylamine 30:70:0.02
Flow rate: 1
Injection volume: 10-100
Detector: UV 310

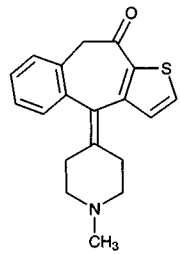
CHROMATOGRAM
Retention time: 10.7 (R), 19.5 (S)
Internal standard: ketorolac

OTHER SUBSTANCES
Extracted: tiaprofenic acid

KEY WORDS
derivatization; chiral; plasma; ketorolac is IS

REFERENCE
Vakily,M.; Jamali,F. Pharmacokinetics of tiaprofenic acid in humans: Lack of stereoselectivity in plasma using both direct and precolumn derivatization methods, *J.Pharm.Sci.*, **1996**, *85*, 638–642.

Ketotifen



Molecular formula: $C_{19}H_{19}NOS$
Molecular weight: 309.43
CAS Registry No.: 34580-13-7, 34580-14-8 (fumarate)
Merck Index: 5319
Lednicer No.: 3 239

SAMPLE
Matrix: blood, tissue
Sample preparation: Blood. Dilute 1 mL plasma with 100 μL 1 M pH 9 phosphate buffer and 100 μL water, add 8 mL diethyl ether, extract. Evaporate the organic layer and dissolve the residue in 400 μL mobile phase. Inject 200 μL aliquot. Tissue. Homogenize the brain with 2

fold the weight of water. Dilute 1500 μL brain homogenate with 500 μL 1 M pH 9 phosphate buffer, add 8 mL diethyl ether, extract. Evaporate the organic layer, dissolve the residue in 400-500 μL mobile phase. Inject a 200 μL aliquot.

HPLC VARIABLES
Column: 250 × 6 Intersil ODS-2
Mobile phase: MeCN:0.018% TFA 20:80 (plasma), MeCN:0.018% TFA 15:85 (tissue)
Column temperature: 40
Flow rate: 0.7
Injection volume: 200
Detector: UV 300

CHROMATOGRAM
Limit of quantitation: 5 ng/mL (plasma), 40 ng/mL (brain)

KEY WORDS
brain; cat; mouse; pharmacokinetics; plasma; rat

REFERENCE
Kato,M.; Nishida,A.; Aga,Y.; Kita,J.; Kudo,Y.; Narita,H.; Endo,T. Pharmacokinetic and pharmacodynamic evaluation of central effect of the novel antiallergic agent betotastine besilate, *Arzneimittelforschung*, **1997**, *47*, 1116–1124.

SAMPLE
Matrix: solutions

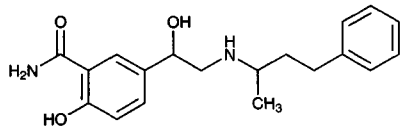
HPLC VARIABLES
Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: k' 14.72

OTHER SUBSTANCES
Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE
Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

Labetalol



Molecular formula: $C_{19}H_{24}N_2O_3$
Molecular weight: 328.41
CAS Registry No.: 36894-69-6, 32780-64-6 (HCl)
Merck Index: 5341
Lednicer No.: 3 24; 4 20